14 GAS CHROMATOGRAPHY

14.1 Objectives

14.1.1 To familiarize the trainee with the theory and application of gas chromatography in drug analysis

14.1.2 To familiarize the trainee with the GC instrumentation and software used in the laboratory

14.2 Modes of Instruction

14.2.1 Self-directed study through reading assignments

14.2.2 Presentations and demonstrations

14.2.3 Study questions

14.2.4 Practical exercise

14.3 Reference

14.3.1 Moffat, A. C., editor. *Clarke's Isolation and Identification of Drugs*. London: The Pharmaceutical Press, 1986, pp. 178-200.

14.3.2 *Basic Training Program for Forensic Chemists*, U.S. Department of Justice, Drug Enforcement Adminstration, Office of Science and Technology, pp. 5-31 through 5-47.

14.3.3 DFS Controlled Substances Procedures Manual, Gas Chromatography Section.

14.3.4 Stafford, David T., Ph.D. "Forensic Capillary Gas Chromatography", in Saferstein, Richard, Ph.D., editor. *Forensic Science Handbook, Volume II.* Englewood Cliffs, N. J.: Prentice Hall, 1988, pp. 38-67.

14.3.5 Hyver, K.J., Sandra, P., editor. *High Resolution Gas Chromatography, Third Edition.* Hewlett Packard Company, 1989.

14.3.6 Rood, Dean, *A Practical Guide to the Care, Maintenance, and Troubleshooting of Capillary Gas Chromatographic Systems, 3rd ed.,* Wiley-VCH, New York, 1999.

14.3.7 Regis Chemical Company. *A User's Guide to Chromatography*. Morton Grove, IL: Regis Chemical Company, 1976, pp. 20-114.

14.3.8 Hewlett Packard and Agilent Technologies GC instrument manuals.

14.3.9 Pierce, A. E., *Silylation of Organic Compounds*, Pierce Chemical Company, Rockford, IL 1968.

14.4 Assignments

14.4.1 Completion of required reading assignments (14.3.1, 14.3.3)

14.4.2 Study questions and practical exercises

14.5 Study Questions

14.5.1 What is gas chromatography?

14.5.2 What types of information are obtained from GC?

14.5.3 Draw a schematic diagram for a GC and describe the purpose of each component.

14.5.4 Define the following terms:

- Resolution
- Carrier gas
- Mobile phase
- Stationary phase
- Partition
- Volatility
- Distribution coefficient
- Retention time
- Retention index
- Linear velocity
- Flow rate
- Injection port
- Flame ionization detector
- Derivatization
- Internal standard

14.5.5 Describe the differences between the solid support used in packed columns and that used in a capillary column GC system.

14.5.6 What general criteria should all stationary phases possess? How do they differ between packed and capillary systems?

14.5.7 What general criteria should all mobile phases possess?

14.5.8 Besides the stationary phase, what factors influence column selection for a given GC application?

14.5.9 What determines the appropriate column diameter for a given GC system? The appropriate length? Why are packed column lengths limited to a maximum of 3 meters?

14.5.10 Describe how the following concepts affect GC separation between components:

- Solubility
- Boiling point
- Intermolecular forces

14.5.11 Describe the following types of capillary columns:

- SCOT
- WCOT
- Fused Silica

14.5.12 What factors influence the “inertness” of a column?

14.5.13 What is the purpose of the polyimide/polyamide coating on a fused silica column?

14.5.14 What is the difference between a bonded and a cross-linked phase? What advantages does a bonded/cross-linked phase column possess?

14.5.15 How are packed columns or liners deactivated after installation? How does it work?

14.5.16 What is column bleed?

14.5.17 When and why are columns conditioned? Describe the process.

14.5.18 What factors govern the operating temperature of a given GC column? What are the upper and lower temperature limits for the following liquid phases? What is the effect of operating above or below these limits?

- SE-30
- Carbowax (both bonded and non-bonded)
- HP-1 (for capillary columns)
- HP-5 MS (for capillary columns)

14.5.19 Define:

- retention time (T_R or tR),
- relative retention time (RRT),
- retention volume,
- unretained retention time (t_m)
- corrected or adjusted retention time (t'_R or t'R)
- phase ratio (β)
- selectivity (α)

14.5.20 Define partition coefficient (K)? What is it a function of? How does it relate to equilibrium? What is meant if K = 1?

14.5.21 What is the partition ratio/capacity ratio (k)? How does it relate to retention time?

14.5.22 Define the following:

- theoretical plate (n)?
- effective theoretical plate (N)?
- theoretical plate height /height equivalent to a theoretical plate (H or HETP)
- height equivalent to an effective theoretical plate (H or HEETP)
- average linear gas velocity (μ)

14.5.22.1 What is a good value for the HETP? And why?

14.5.22.2 How is the # of N related to column efficiency?

14.5.23 Define Resolution (R).

14.5.23.1 What is chromatographic resolution a function of?

14.5.23.2 Why is resolution not the best measure of column efficiency and column performance?

14.5.24 Discuss the effects of column i.d. and stationary phase film thickness with respect to sample capacity, column efficiency, relative retention times and resolution.

14.5.25 Diagram and explain the Van Deemter plot. Why does the drug lab use Helium as a carrier gas?

14.5.26 What two factors influence the relative retention time of two components?

14.5.27 What is the Kovats retention index (I)? What does it mean if I = 650?

14.5.28 Define Separation Number/Trennzahl Number (TZ). What does it mean if TZ = 3?

14.5.29 What affect do the following have on retention time:

- Concentration
- Other compounds in the sample
- Free base/acid form vs. salt form

14.5.30 What should be the minimum retention time of the first eluting component in a sample of one or more components to insure the sample has spent enough time in the liquid phase to achieve reasonable separation?

14.5.31 Discuss the relationship between geometry, pressure drop, column capacity, resolution, sensitivity, speed and column bleed with respect to capillary columns and their packed column counterparts.

14.5.32 Discuss the sample introduction of gases and vapors, volatile liquids and solids into a GC.

14.5.33 What is meant by flash vaporization?

14.5.34 Describe the proper manual injection technique.

14.5.35 What factors govern the amount of sample to be injected? How much sample/component can the average capillary column hold? What factors influence this?

14.5.36 What temperature should the injection port be under normal circumstances and why?

14.5.37 What type of septa are recommended for GC work and why?

14.5.38 What are the differences and purposes of “split” injection, “splitless” injection, “on-column” injection, and “direct-on-column” injection?

14.5.39 What is an injection port liner? What is it made of? Why is it used? Describe the packing process including the materials used.

14.5.40 What is a “split ratio” and how is it calculated?

14.5.40.1 What factors govern the use of a particular split ratio (100:1 vs. 50:1)?

14.5.40.2 What is meant by linear split, why is it desirable and how is it achieved?

14.5.41 Describe the “solvent effect”?

14.5.41.1 How is it done and why is it important?

14.5.41.2 What factors affect the efficiency of the solvent effect?

14.5.41.3 Define the solvent effect with respect to the equation $K = \beta k$.

14.5.42 What is meant by “cold trapping” and how is it done?

14.5.43 Why is it necessary to regulate the carrier gas flow?

14.5.43.1 How is this done?

14.5.43.2 What factors influence the optimum flow rate for a given carrier gas?

14.5.43.3 If the carrier gas is too fast or too slow how will it affect the peak shapes of your sample components?

14.5.43.4 How will it affect the detector?

14.5.44 Discuss the various detector types (especially Thermal Conductivity, Flame Ionization (FID) and Electron Capture) with respect to the following:

- How does each work?
- Carrier gas requirements
- Sensitivity
- Temperature requirements
- Stability
- Insensitivities
- Advantages/disadvantages with respect to organic drug analysis

14.5.45 What is “make-up” gas?

14.5.45.1 How and why is it used?

14.5.45.2 What determines which gas will be used as a make-up gas?

14.5.46 Explain the following statement: “response is proportional to the number of carbon atoms in the sample”.

14.5.46.1 What type(s) of detector is this statement applicable to?

14.5.46.2 What is meant by “mass-flow” detector?

14.5.47 What is an attenuator and how and why is it used? Is it linear?

14.5.48 What is a recorder and how does it differ from currently used data acquisition devices?

14.5.49 What types of compounds should be included in a test mixture used to assess chromatographic performance? Why would these compounds be included and what would each be designed to evaluate?

14.5.50 What types of GC’s (model, manufacturer, etc.) does the drug laboratory use?

14.5.50.1 What types of injection ports, carrier gases, flows, columns and detectors does each GC incorporate?

14.5.50.2 What type of integrator (s) does the drug laboratory use? Are they mechanical or electronic?

14.5.51 Outline a logical troubleshooting schematic for isolating the source of a GC system problem.

14.5.52 What three things can cause insufficient gas flow through a GC system?

14.5.53 Describe how to change the septum on each of the GC’s.

14.5.53.1 What are some of the problems encountered when a septum is too tight or too loose?

14.5.54 What are some of the common causes and remedies for the following GC system problems:

- No peaks

- Solvent peak only
- Baseline drift or unstable baseline
- Ghost peaks
- Tailing peaks
- Leading peaks
- Split peaks
- Baseline rise before or after a peak
- Baseline drop after a peak
- Retention time shift

14.5.55 Describe the preventative maintenance schedule and QA/QC procedures performed on the GC's.

14.5.56 Discuss the operation of an autosampler.

14.5.57 What is "needle discrimination" and how is it corrected?

14.5.58 What is gas saver and how is it used?

14.5.59 What is EPC? Explain the difference between constant flow and constant pressure.

14.5.60 Draw a diagram of the injection port and illustrate the carrier gas flow throughout for both split and splitless injections.

14.5.61 Explain how derivatization is performed, including why it is used sometimes for analysis.

14.5.62 Describe the internal standard method of quantitation. How accurate is the method generally?

14.5.63 What is the mathematical formula for calculating purity? Define each variable.

14.5.64 If two drug compounds were to co-elute on the GC, what could be done to resolve the peaks?

14.5.65 Explain as to a jury how a GC operates.

14.6 Practical Exercise

14.6.1 Write a method for the GC which creates a program which will perform the following:

- Injector and detector temperatures: 280°C
- Oven temperature: 150-250°C, 10°C per minute, initial hold of 2 minutes
- Total run time: 20 minutes
- Split ratio: 50:1
- Column flow rate: 1 mL/min

14.6.1.1 Now inject a mixture of cocaine and propoxyphene and see if the two compounds resolve. If not, change the method one parameter at a time until they are resolved.

14.6.2 Inject the following standards on the GC and describe their peak shapes:

- Amphetamine sulfate in $CHCl_3$
- Amphetamine base in $CHCl_3$
- Flash-derivatized amphetamine acetate in $CHCl_3$

14.6.3 Perform the derivatization procedure for the differentiation of d- and l- methamphetamine as outlined in the Virginia Division of Forensic Science Drug Analysis Procedures Manual.

14.6.4 Obtain an unknown sample of cocaine or heroin from the TC and perform a quantitative analysis.

14.7 Modes of Evaluation

14.7.1 Written examination

14.7.2 Court exercise (mini-mock trial)

♦ End